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Acidification in the in vitro perfused tubule

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The kidneys' major functions in regulating the acid-base balance of body fluids are *(1)* to conserve plasma bicarbonate and *(2)* replenish plasma bicarbonate lost in titration with nonvolatile acids. The proximal nephron reabsorbs the bulk of filtered bicarbonate, and, thus, predominates in the first function. The distal nephron forms the bulk of the net acid excreted, and, thus, predominates in the second function. A single mechanism, hydrogen ion secretion, is responsible for both functions. The renal tubular cells produce hydrogen and bicarbonate ions from the dissociation of intracellular carbonic acid catalyzed by intracellular carbonic anhydrase. The hydrogen ions are secreted into the lumen and simultaneously the bicarbonate ions move into the peritubular plasma. It is the details of the mechanism of hydrogen ion secretion and the control of hydrogen ion secretion which differ between the proximal and distal nephron segments. The purpose of this review is to describe these differences as elucidated by the technique of isolated perfused nephron segments.

It should be noted at the outset that a great many of the advances in this field have been made in the last 5 years and can be ascribed to the development of microcalorimetry [1]. This technique measures picomole amounts of total carbon dioxide, which include bicarbonate, carbonic acid, and dissolved carbon dioxide. At pH 7.4, 95% of the total carbon dioxide in physiologic fluids is bicarbonate, and, thus, the measured quantity will be referred frequently to as bicarbonate. The advantage of microcalorimetry over conventional pH electrodes is predominately at bicarbonate concentrations in the range of 25 mM. In this range small pH changes (less than 0.1 U) are associated with large differences in bicarbonate concentration (4 to 5 mM).

Proximal nephron

The great bulk of renal hydrogen ion secretion takes place in the proximal nephron to reabsorb filtered bicarbonate. In normal acid-base states more than 98% of all hydrogen ions secreted by the renal tubules are involved in this function. The technique of isolated tubule perfusion has contributed greatly to the understanding of the mechanism of hydrogen ion secretion in the proximal nephron and to our knowledge of the factors which control hydrogen ion transport in this segment.

Bicarbonate reabsorption. The isolated proximal tubule is capable of reabsorbing bicarbonate and generating considerable bicarbonate concentration gradients between the tubular lumen and the bath. This was inferred initially from increases in the collected fluid chloride concentration [2] and then first directly measured using microcalorimetry by Warnock and Burg [3]. Rates of bicarbonate absorption are shown in Table 1. The greatest rates of bicarbonate absorption are seen in the late superficial and juxtamedullary proximal convoluted tubule (PCT), 80 to 100 pmoles/mm · min [4–7]. Lesser rates are seen in the early superficial PCT, 40 pmoles/mm · min [6], in the proximal straight tubule (PST), 24 to 30 pmoles/mm · min [8, 9], and in the juxtamedullary PCT of newborn rabbits, 24 pmoles/mm · min [10]. In both the PCT and the PST, bicarbonate transport can be described by a pump-leak system with a steady-state luminal bicarbonate concentration of 6 to 8 mM [4, 8, 9, 11]. The pump-leak system can be divided into an active transcellular absorptive component and a passive leak component.

Active transcellular absorptive component. The active step in the hydrogen secretory mechanism is located at the luminal cell membrane. Measurements of intracellular pH in isolated tubule suspensions show that the inside of the cell is alkaline, approximately pH 7.5 [12], and that the potential difference (PD) is interior negative by 50 to 70 mV [13]. The transport of hydrogen ions from cell to lumen occurs against an unfavorable electrochemical gradient, and, thus, is an active process requiring the expenditure of metabolic energy.

Two alternative mechanisms for secreting hydrogen ions across the luminal cell membrane have been hypothesized: *(1)* Hydrogen ions are extruded in exchange for the sodium ions, with the energy provided by the sodium gradient. *(2)* Hydrogen ions are extruded alone, with the energy provided directly by metabolism. In the proximal tubule the in vivo evidence identifying the mechanism, and thus the energy source, has been conflicting. Some micropuncture studies have shown that hydrogen ion secretion depends, in part, on sodium transport; some in vivo electrophysiologic studies have indicated electrogenic hydrogen ion secretion. In the in vitro isolated proximal tubule, however, the evidence identifying the mechanism of hydrogen secretion and the source of the energy utilized to secrete hydrogen ions is consistent. Hydrogen ion secretion occurs, at least in part, by an electroneutral exchange process with sodium ions [14, 15]. The energy for the uphill movement of hydrogen ions from cell to lumen is provided by the favorable electrochemical gradient for the downhill movement of sodium

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Table 1. Rates of bicarbonate absorption

Segment	Rate *pmoles/mm · min*	Reference
Proximal convoluted tubule	80 to 100	4 to 7
Proximal straight tubule	20 to 30	3, 8, 9
Thin limbs	ND	
Thick ascending limb	0	67, 68, 69
Distal convoluted tubule	ND	
Connecting tubule	ND	
Cortical collecting tubule	±5	84, 85
Medullary collecting tubule	20	97, 98, 100

Abbreviation: ND, not determined.
[a] Both absorption [84] and secretion [85] have been observed.

ions from lumen to cell. The low intracellular sodium concentration is maintained by the sodium-potassium ATPase pump system. Support for this model includes, first, the observation that experimental maneuvers which interfere with sodium absorption, such as sodium removal, bath ouabain, or removal of bath potassium, dramatically inhibit bicarbonate absorption (Sasaki, Berry, and Rector, to be published) [4, 11]. Second, the neutral characterization of proximal acidification is supported by the finding that in the absence of organic solutes, sodium bicarbonate absorption does not generate an independent lumen-positive transepithelial PD [16].

It could, however, be argued that the dependence of bicarbonate absorption on sodium absorption is not directly through a sodium-hydrogen exchanger but is secondary through an effect of sodium transport on intrarenal P_{CO_2} or energy metabolism. Recently, more direct evidence for a sodium-hydrogen exchanger in the PCT has been obtained. First, the hydrogen ion efflux rate from an acidic perfusate, which is dependent on the lumen to cell sodium gradient and is independent of metabolism, was stimulated when cell sodium concentration was increased with ouabain [17]. Second, when luminal and basolateral sodium were replaced, intracellular pH failed to recover from an acid load in salamander tubules [18]. Third, amiloride, an inhibitor of sodium-hydrogen exchange in brush-border vesicles [14, 19], inhibits both hydrogen ion efflux from an acidic perfusate [17] and the recovery of intracellular pH following an acid load [18]. These data are good evidence for a reversible sodium-hydrogen ion exchanger in the luminal cell membrane of both rabbit and salamander, and in the salamander basolateral cell membrane.

Concerning the isolated tubule, controversy exists regarding whether all proximal acidification occurs via a sodium-hydrogen exchanger or whether some hydrogen secretion might occur independent of sodium absorption. The proportion of proximal bicarbonate reabsorption dependent on sodium absorption and, thus, attributable to a sodium-hydrogen exchange, can be determined from the degree of inhibition of bicarbonate absorption following maneuvers which completely inhibit sodium transport. In early studies complete inhibition of sodium transport with ouabain or with bath potassium removal caused the bicarbonate concentration gradient developed at slow flow rates to be reduced from approximately 15 to 5 mM rather than to zero [4, 11]. The residual acidification was attributed to a sodium-independent mechanism of acidification. In one study, however, sodium removal totally inhibited the rate of bicarbonate absorption [4]. Recently, the dependence of bicarbonate absorption on sodium transport has been re-examined using bath potassium removal to inhibit sodium transport (Sasaki, Berry, and Rector, to be published). After exhaustively dialyzing albumin against potassium-free solutions (Sasaki, Berry, and Rector, to be published) were able to achieve a bath potassium concentration of 0.04 mM and a reduction in bicarbonate absorption of 90 ± 4% of control. To determine if these data were significantly different from 100% inhibition, we examined the ability of the PCT to generate a bicarbonate concentration gradient at slow flow rates when bathed in 0.04 mM potassium. The bicarbonate concentration gradient generated (0.4 to 0.7 mM) was not different from that found when the perfusate was inserted directly into the collection pipette in the absence of a tubule. These studies show that virtually all of proximal bicarbonate absorption is dependent on sodium transport.

Sodium bicarbonate absorption itself does not generate a transepithelial PD [16]. Accordingly, it is not associated with net current flow through the paracellular shunt pathway or with net current flow across the apical or basolateral cell membranes. The neutral transport of sodium bicarbonate across the apical cell membrane is achieved by the sodium-hydrogen antiporter. The mechanism for the neutral movement of sodium bicarbonate across the basolateral cell membrane is less well understood. It is believed generally that sodium is transported across the basolateral cell membrane through the sodium-potassium ATPase pump system. The nature of bicarbonate exit will depend on the electrogenicity of the sodium-potassium ATPase pump system. Recent evidence suggests that the pump is electrogenic [20], probably with a coupling ratio of three sodiums for two potassiums [21]. Neutral sodium bicarbonate transport across the basolateral cell membrane requires that any cation current flow be balanced by an equal anion current flow. Therefore, if the pump is electrogenic, at least some of the bicarbonate must exit conductively. Since intracellular bicarbonate concentration is approximately equal to plasma [22], the driving force for bicarbonate exit is equal to the cell membrane potential, about −60 mV [13, 23]. To date, however, it has not been possible to unequivocally demonstrate a bicarbonate conductance in the basolateral cell membranes of the mammalian proximal tubule [13, 23]. The principal difficulty encountered in attempting to demonstrate a bicarbonate conductance is that lowering peritubular bicarbonate concentration must change the pH of either the intracellular or the peritubular compartment, and such pH changes have been shown to modify the potassium conductance of the basolateral cell membrane [13, 23]. Evidence favoring a conductive exit step for bicarbonate, however, has been found in the salamander proximal tubule [18]. For an electrogenic sodium-potassium ATPase pump system, the amount of bicarbonate which exits conductively will depend on the mechanism by which potassium, transported into the cell by the sodium-potassium ATPase, exits. The mechanism of potassium exit is important because it might contribute to cation current flow across the basolateral cell membrane. If all of the potassium exits conductively, all of the sodium transported across the basolateral cell membrane is current carrying (either as sodium through the pump or as potassium diffusion), and, therefore, all of the bicarbonate must exit conductively. This possibility for sodium bicarbonate transport across the basolateral cell membrane is depicted in

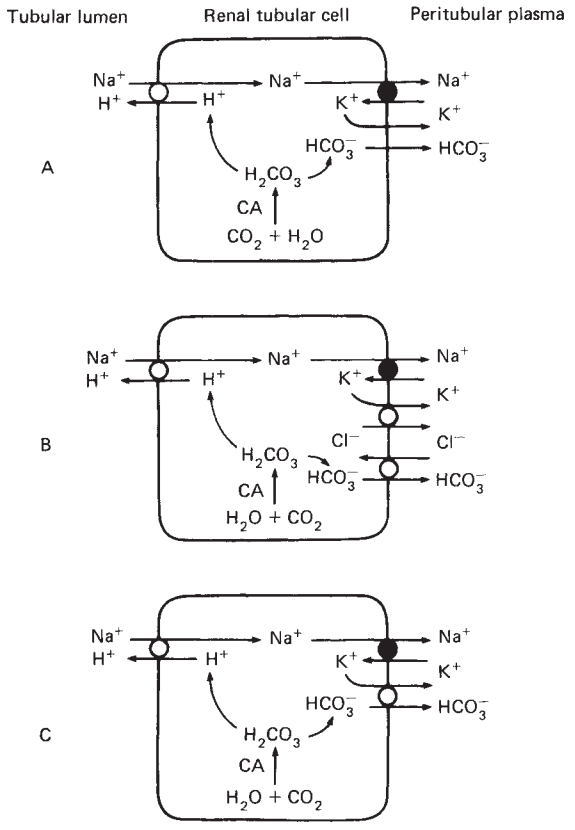

Fig. 1. *Possible mechanisms of bicarbonate ion transport across the basolateral cell membrane of the proximal tubule. Open circles* denote ion exchange mechanisms responsive to chemical concentration gradients. *Closed circles* denote ion exchange mechanisms requiring direct expenditure of metabolic energy. *Arrows* represent simple diffusion responsive to the electrochemical gradient. In the PCT sodium bicarbonate absorption is neutral, therefore, net current does not cross either the luminal or peritubular cell membrane. The mechanism of bicarbonate ion exit depends on the cation current mechanism induced by sodium exit. In panel **A** all sodium exit associated with sodium bicarbonate transport carries current, in part through the Na^+-K^+ ATPase pump system, and, in part, via potassium diffusion, and to maintain electrical neutrality, all bicarbonate ion exits conductively. In panels **B** and **C** sodium exit associated with sodium bicarbonate transport does not carry current, the Na^+-K^+ ATPase pump is neutral, potassium exit is neutral, and all bicarbonate ion exits neutrally. In panel **B** bicarbonate ion exits in exchange for chloride. In panel **C**, potassium bicarbonate exits. It should be noted in panels **B** and **C** that if the Na^+-K^+ ATPase pump system is electrogenic, or if any potassium diffusion exists, then an equivalent portion of bicarbonate ion exit must be conductive, as in panel **A**.

panel A of Figure 1. Alternatively, if potassium exits via a neutral mechanism, then the associated bicarbonate must also exit neutrally. Potassium exit via a neutral potassium chloride symporter is depicted in panel B of Figure 1. Under these conditions, a neutral bicarbonate exit might be effected by a chloride-bicarbonate antiporter. Evidence against a chloride-bicarbonate antiporter has been obtained by Burg and Green [4]. Removal of luminal and peritubular chloride, and replacement with nitrate, does not influence bicarbonate absorption in the PCT. These studies, however, can be criticized because nitrate appears to have a high affinity for the chloride-bicarbonate exchanger, at least in intestine brushborder membrane vesicles [24]. Finally, a neutral potassium exit with chloride-independent, bicarbonate absorption might be effected by a neutral potassium bicarbonate symporter, as depicted in panel C of Figure 1. Also in Figure 1, panel A is a complete cell model assuming the sodium-potassium ATPase pump system is electrogenic. Panels B and C are complete cell models if the sodium-potassium ATPase is neutral and are incomplete if the sodium-potassium ATPase is electrogenic. For an electrogenic sodium-potassium ATPase pump system with a coupling ratio of three sodiums to two potassiums [21], a minimum of one third of the bicarbonate leaving the cell must exit via a conductive pathway to balance the sodium current through the pump. Furthermore, if there is any conductive potassium exit, an additional component of conductive bicarbonate exit would be required.

Despite the facts that essentially all bicarbonate absorption in the rabbit proximal tubule can be attributed to electroneutral sodium-hydrogen exchange and bicarbonate absorption in the absence of luminal organic solutes does not generate an independent lumen-positive transepithelial PD, maneuvers modifying acidification can alter the transepithelial PD in the presence of organic solutes. The cotransport of sodium with neutral organic solutes, such as glucose and alanine, is responsible for the lumen-negative PD in PCT perfused with ultrafiltrate [25, 26]. In the presence of luminal organic solutes, stimulation of acidification by increasing the ambient carbon dioxide tension [6] causes the lumen-negative PD to depolarize by approximately 1 mV. Reductions of acidification by carbonic anhydrase inhibitors [6, 16], by bath SITS [16], or by decreasing the ambient carbon dioxide tension [6], causes the lumen-negative PD to hyperpolarize by approximately 1 mV. These electrical effects of acidification observed in the presence of organic solutes suggest that there is an interaction between the rate of acidification and the rate of other electrogenic transport processes [16] or an effect of cell pH on transepithelial PD, perhaps mediated by its effect on basolateral potassium conductance [20, 23].

Carbonic anhydrase functions to facilitate bicarbonate absorption in the proximal tubule in at least two and perhaps three important ways: First, inside the cell, carbonic anhydrase catalyzes the hydration of carbon dioxide to form carbonic acid which subsequently dissociates to form hydrogen ions that are secreted into the tubular lumen and bicarbonate ions which diffuse into the peritubular plasma. Second, in the brush border of the luminal membrane, carbonic anhydrase catalyzes the dissociation of luminal carbonic acid into carbon dioxide and water. Early micropuncture studies suggested that about half of proximal tubule bicarbonate absorption remained after administration of the carbonic anhydrase inhibitor acetazolamide. These studies implied that about half of bicarbonate absorption was carbonic anhydrase-dependent and half was carbonic anhydrase-independent. As a result of the large portion of carbonic anhydrase-independent bicarbonate absorption, other mechanisms of bicarbonate absorption were proposed, such as direct bicarbonate absorption and carbonic acid recycling. It was not until the advent of studies in the isolated proximal tubule that a quantitatively more important role for carbonic anhydrase in proximal bicarbonate absorption was appreciated. In the PST, the addition of 10^{-5} M acetazolamide to the perfusate and bath caused the collected bicarbonate concentration at slow flow rates to rise from 25 to 32 mM [11]. The rise in concentration

was not significantly different from the rise in the inulin concentration, suggesting that bicarbonate absorption was inhibited totally. In other words, there was no evidence for carbonic anhydrase-independent bicarbonate absorption in the PST. Studies in the isolated PCT have shown that in the presence of bath acetazolamide, the collected concentration of bicarbonate was approximately equal to the concentration perfused [6]. In these studies, the rate of carbonic anhydrase-independent bicarbonate absorption depended on the rate of volume absorption and was generally between 10 and 30 pmoles/mm · min. A portion of this acetazolamide-insensitive, carbonic anhydrase-independent bicarbonate flux might have been eliminated if the drug had also been present in the perfusate. Burg and Green [4] found a greater effect on volume absorption of perfusate and bath acetazolamide than of bath acetazolamide alone. Since approximately 20% of proximal bicarbonate absorption is carbonic anhydrase-independent in vivo, it seems likely that a small percentage of bicarbonate absorption may remain following carbonic anhydrase inhibition in the in vitro PCT. The amount, however, is at most 30 pmoles/mm · min. The uncatalyzed rate of reaction for the hydroxylation of carbon dioxide to bicarbonate depends on cell volume and in the PCT can account for approximately 15 pmoles/mm · min [27]. Furthermore, since the PD is lumen-negative, some carbonic anhydrase-independent bicarbonate absorption might be attributable to passive diffusion [28, 29]. The uncatalyzed rate, or diffusion, can probably account for the carbonic anhydrase-independent bicarbonate absorption in the isolated PCT. Studies are needed to examine the carbonic anhydrase-independent bicarbonate absorption in superficial and juxtamedullary PCT. The bicarbonate ion permeability is greater in superficial than in juxtamedullary PCT [28, 29], indicating that there may be more carbonic anhydrase-independent, diffusive bicarbonate absorption in superficial than in juxtamedullary PCT. The reason for the discrepancy between the PST and the PCT is unclear. It is probably partially due to the absence of luminal inhibitor in PCT, an underestimate of the perfusate bicarbonate concentration in PST, and the low bicarbonate permeability in the chloride selective superficial PST.

Passive leak component. The passive movement of any of the components of the bicarbonate-carbon dioxide buffer system can influence bicarbonate absorption. The values for carbon dioxide, bicarbonate ion, and hydrogen ion permeability have been determined explicitly in the isolated proximal tubule. From these values the significance of the leak component to net bicarbonate absorption can be approximated. The transepithelial route of the passive leak component might be either transjunctional or transcellular.

In the proximal tubule, secreted hydrogen ions react with luminal bicarbonate to form carbon dioxide. The carbon dioxide is reabsorbed, presumably by diffusing down its concentration gradient. In the rabbit proximal tubule, the permeability of carbon dioxide is so high, approximately 650×10^{-5} cm/sec, that only trivial concentration gradients of less than 4 mm Hg are required to account for the observed rates of bicarbonate absorption [30, 31]. From these data one would not predict that there is a substantial difference in carbon dioxide tension between tubular fluid and peritubular plasma in vivo. Since carbon dioxide is highly lipid soluble and the cell cytoplasm contains carbonic anhydrase, it is probable that the bulk of the carbon dioxide permeates via the transcellular pathway.

Proximal tubular epithelial hydrogen ion permeabilities have been determined from the rate of hydrogen ion transport in response to a transepithelial pH gradient. Under these conditions, however, there are several mechanisms, other than diffusion, by which hydrogen ions or their equivalent can cross the proximal tubular epithelium. These mechanisms include sodium-hydrogen exchange, chloride-hydroxyl exchange and nonionic diffusion of buffers such as lactate or acetate [31]. Consequently, the determination of diffusional hydrogen ion permeability should be done in the absence of sodium, chloride, and permeable buffers. This has not been done yet. Thus, the present value for hydrogen ion permeability, 0.21 to 0.26 cm/sec [17, 32], represents an upper limit. However, even this seemingly huge permeability is incapable of generating significant fluxes because of the extraordinarily low hydrogen concentrations in physiologic fluids. If the bath pH is 7.4 and the luminal pH is 6.8, this hydrogen ion permeability will only contribute 2 to 3 pmoles/mm · min of hydrogen ion leak from lumen to bath, and, as such, will not substantially shunt the active transcellular component. Since a rheogenic hydrogen ion flux can be demonstrated in renal brushborder membranes [33], at least a portion of this transepithelial hydrogen ion permeability is probably transcellular.

On the other hand, bicarbonate ion permeability appears to be quite small, approximately $1.0\ 10^{-5}$ cm/sec [28, 29, 34], but it may be an important shunt of active transcellular hydrogen ion secretion. The reason bicarbonate ions can shunt, but hydrogen ions cannot, is because the bicarbonate ion concentration is in the millimolar range, whereas the hydrogen ion concentration is in the nanomolar range. Whether or not bicarbonate ion permeability does contribute to shunting, however, depends on its actual magnitude. Bicarbonate ion permeability has not been determined precisely for the same reasons that hydrogen ion permeability has not. There are mechanisms other than simple diffusion that could be involved in bicarbonate transport, such as sodium-hydrogen and chloride-hydroxyl exchange.

Two techniques have been used to determine bicarbonate ion permeability: First, microcalorimetry has been used to measure total carbon dioxide fluxes in response to a bicarbonate ion concentration difference. These data give a bicarbonate ion permeability, ranging from 1.7 to $2.3\ 10^{-5}$ cm/sec [7, 28]. Second, isotopic isethionate permeability and electrophysiologic bicarbonate to isethionate permeability ratios have been used to calculate bicarbonate ion permeability. These data give 0.4 to $1.3\ 10^{-5}$ cm/sec [29, 34]. If there is a 15 mM bicarbonate ion concentration gradient across the proximal tubule, these permeabilities will give bicarbonate fluxes of between 5 and 32 pmoles/mm · min. The lower values are more likely to be representative of the backleak through the paracellular pathway since they were determined using electrophysiologic techniques which presumably measure paracellular properties [35]. In summary, bicarbonate ion backleak may represent 5 to 30% of the measured net fluxes. Thus, the rate of active hydrogen ion secretion is 105 to 130% of the measured net fluxes.

Control of proximal bicarbonate absorption

Acid-base determinants. The isolated tubule preparation has offered a unique opportunity to examine the effect of acid-base factors, such as luminal and peritubular bicarbonate concentration and P_{CO_2}, on bicarbonate absorption. This technique allows perfusate and bath bicarbonate concentrations to be

changed independently and excludes the effects of other physiologic control factors that often interfere with the interpretation of in vivo studies, such as plasma potassium concentration, extracellular volume status, and hormones.

Increasing luminal bicarbonate concentration in PCT from 25 to 40 mM without changing either peritubular bicarbonate concentration (25 mM) or P_{CO_2} (40 mm Hg) stimulated bicarbonate absorption from 96 to 135 pmoles/mm · min [7]. Conversely, increasing peritubular bicarbonate concentration from 25 to 40 mM without changing luminal bicarbonate concentration (40 mM) or P_{CO_2} (40 mm Hg) inhibited bicarbonate absorption from 139 to 76 pmoles/mm · min [7]. Approximately 80% of both the stimulation induced by increasing luminal bicarbonate and the inhibition induced by increasing peritubular bicarbonate could be accounted for by an effect on the active transcellular component of bicarbonate absorption [7].

The effect of luminal and peritubular bicarbonate concentrations on proximal bicarbonate absorption are in accord with in vivo data [36, 37] and can be interpreted in terms of the current view of bicarbonate absorption. An increase in luminal bicarbonate concentration raises the luminal pH and reduces the hydrogen ion concentration gradient against which the sodium-hydrogen exchanger operates. As a result, the rate of hydrogen secretion, and thus bicarbonate absorption, increases [7]. The effect of luminal bicarbonate can be explained in terms of its influence on the chemical gradient for hydrogen ions across the luminal cell membrane. If a bicarbonate exit is via a neutral mechanism as depicted in panels B and C of Figure 1, then the effect of peritubular bicarbonate might also be explained by its effect on the chemical gradient for bicarbonate across the basolateral cell membrane. However, if it is electrogenic as depicted in panel A of Figure 1, then the effect of peritubular bicarbonate probably cannot be explained by its effect on the electrochemical gradient. Biagi, et al [13] have shown that peritubular alkalinity hyperpolarized the basolateral membrane PD. Therefore, when peritubular bicarbonate is increased, the electrochemical driving force for bicarbonate diffusion might be constant. In this case, an effect of peritubular bicarbonate on basolateral membrane permeability might be the primary event. Whatever the primary effect of luminal and peritubular bicarbonate concentrations, each will cause a change in intracellular pH and thereby transmit the effect to the contralateral cell membrane. In the steady-state, changes in the rate of acidification must be due to alterations in the acidification processes at both the luminal and basolateral cell membranes.

Acute changes in P_{CO_2} in vitro are associated with dramatic effects on bicarbonate absorption from the PCT. Increases in P_{CO_2} from 40 to 70 mm Hg stimulated bicarbonate absorption between 12 and 60% [6, 7]. In contrast, a decrease in P_{CO_2} from 40 to 15 mm Hg inhibited proximal bicarbonate absorption by 70 to 90% [6]. The mechanism by which alterations in P_{CO_2} at constant luminal and peritubular bicarbonte concentration affects proximal bicarbonate absorption is not clear. Changing P_{CO_2} should similarly modify the pH of the luminal, peritubular and intracellular compartments, and, thus, not substantially affect the gradient either for hydrogen ion transport across the luminal membrane or for bicarbonate ion transport across the peritubular membrane. In some manner, increasing P_{CO_2} selectively stimulates, and decreasing P_{CO_2} selectively inhibits, the flux of hydrogen ions across the luminal cell membrane or the flux of bicarbonate ions across the peritubular cell membrane. The mechanism for such a differential effect might be mediated by intracellular pH. For instance, a decrease in intracellular pH might increase basolateral membrane bicarbonate permeability. The physiological significance in vivo of these dramatic in vitro effects of P_{CO_2} on bicarbonate absorption are difficult to evaluate because the normal renal cortical P_{CO_2} is elevated over the systemic plasma P_{CO_2}, and is approximately equal to the highest P_{CO_2} evaluated in vitro, about 65 to 70 mm Hg [38].

Parathyroid hormone. Parathyroid hormone (PTH) appears to be an important regulator of bicarbonate absorption in the isolated perfused superficial PST. Both Iino and Burg [8] and McKinney and Myers [9] find that the steady-state concentration for bicarbonate increases from 8 to 12 and to 23 mM, and that bicarbonate and volume absorption are inhibited by approximately 50%, following the addition of physiological amounts of PTH (0.1 to 1.0 U/ml) to the solutions bathing PST. The data in PCT, however, are contradictory. Iino and Burg [8] find no effect of bath PTH in concentrations up to 5 U/ml on the steady-state bicarbonate concentration. McKinney and Myers [39] find that the steady-state bicarbonate concentration increases from 10 to 20 mM and that bicarbonate and volume absorption are inhibited by approximately 50% following the addition of 0.1 U/ml PTH to the solution bathing PCT.

Studies in the isolated tubule have attempted to define the mechanism of action of PTH on bicarbonate transport in the proximal tubule. Cyclic AMP analogues, such as dibutyryl cyclic AMP and 8 bromo-cyclic AMP, but not 5′-AMP, have been found to mimic the effect of PTH in vitro [9, 39]. Thus, it seems clear that PTH acts via this second messenger. The physiologic effect of PTH and cyclic AMP, however, is controversial. They might decrease net bicarbonate absorption either by inhibiting active transcellular bicarbonate transport from lumen to bath, or by increasing passive paracellular backleak of bicarbonate into the lumen from the bath. In the PCT, the cyclic AMP analogue (8-(p-chlorophenylthio)-cyclic 3′,-5′-adenosine monophosphate) decreased transepithelial PD and volume absorption and increased sucrose permeability [40]. The effect on sucrose permeability was independent of effects on volume absorption. These observations eliminate solute-solvent interaction as the cause of the cyclic AMP analogue effect and indicate that the cyclic AMP analogue directly alters paracellular permeability [40]. An increase in paracellular permeability might increase the backleak of bicarbonate and, thus, reduce bicarbonate absorption. The effect of cyclic AMP on sucrose permeability, however, has not been confirmed [41]. Furthermore, the failure of PTH to affect the flux of bicarbonate into a bicarbonate-free perfusate in the presence of ouabain argues against an effect of PTH on bicarbonate backleak [9, 39]. Unfortunately, these later experiments were not performed in the presence of acetazolamide and as such are not true estimates of proximal bicarbonate permeability [35]. However, a specific effect of PTH and cyclic AMP on active transcellular bicarbonate transport is suggested by the fact that in the absence of bicarbonate, PTH has no effect on volume absorption [42].

The physiological significance of the dramatic in vitro effect of PTH (50% inhibition) to in vivo proximal bicarbonate absorption is unclear. In vivo acute PTH administration results in a modest inhibition of proximal reabsorption in the rat; proximal

bicarbonate absorption is 94% of the filtered load in the absence of PTH and 88% in the presence of PTH [43]. Furthermore, in the dog, chronic PTH results in metabolic alkalosis rather than acidosis [44]. These differences between in vitro and in vivo effects may be due to interactions between PTH, calcium, and vitamin D in vivo [31].

Protein. In clearance studies, reductions in peritubular protein concentration by extracellular fluid volume expansion inhibited whole kidney bicarbonate absorption [45, 46], and increases in peritubular protein concentration by extracellular fluid volume contraction has been suggested to stimulate fractional bicarbonate absorption [47]. It has been assumed that these changes in bicarbonate absorption occur in the proximal tubule and are related to alterations in the colloid osmotic pressure of peritubular plasma and to resultant changes in paracellular bicarbonate permeability [48]. However, recent studies in vitro indicate that removal of peritubular protein from solutions bathing PCT does not alter bicarbonate absorption [5], paracellular bicarbonate permeability [5], or transepithelial specific resistance [49]. In vivo studies in which microperfusion and free-flow techniques were used also did not reveal an influence of extracellular volume expansion on net proximal bicarbonate absorption [36, 50]. Therefore, in contrast to early views, the current view would not consider peritubular protein to be a regulator of proximal bicarbonate absorption.

Potassium. In clearance studies, bicarbonate absorption is related inversely to body potassium stores. Potassium administration depresses and potassium deficiency stimulates bicarbonate absorption even when changes in extracellular fluid volume are taken into account [51]. The change in bicarbonate absorption has been suggested to occur, at least in part, in the proximal tubule in potassium deficiency [52]. However, studies in the in vitro rabbit tubule clearly show that an acute reduction in bath potassium inhibits rather than stimulates bicarbonate absorption in the PCT (Sasaki, Berry, and Rector, to be published) [4, 11]. Lowering bath potassium from 5 to 2 mM resulted in a decrease in bicarbonate absorption from 93 to 72 pmoles/mm · min (Sasaki, Berry, and Rector, to be published). The bath potassium concentration at which bicarbonate absorption was inhibited by 50% was approximately 1 mM. This response of bicarbonate absorption to bath potassium concentration is in keeping with the total dependence of bicarbonate absorption in the PCT on sodium transport. Removal of bath potassium reduces the activity of the sodium-potassium pump system and, thus, inhibits sodium-hydrogen exchange. Failure to find a stimulatory effect of acute reductions in bath potassium in vitro does not address the potential effects of chronic changes in body potassium balance in vivo. However, although the effect of hypokalemia to stimulate ammonia production and thus influence acid-base homeostatis is still appreciated, the importance of changes in either acute or chronic changes in body potassium stores in regulating proximal bicarbonate absorption in vivo are currently in serious doubt [31, 53].

Loop of Henle

Free-flow micropuncture studies in rats [54–56] and dogs [57] determined that luminal bicarbonate and/or pH in the early distal tubule was nearly the same as that at the end of the accessible proximal convoluted tubule. Volume reabsorption in the loop of Henle should have raised the bicarbonate concentration of the fluid delivered to the early distal tubule, so that these findings indicated that luminal acidification and, thus, bicarbonate absorption occurred in the loop [57]. Bicarbonate is absorbed in isolated straight segments of the proximal tubule [3, 11], and accounts, at least in part, for bicarbonate absorption observed in previous in vivo studies of the loop of Henle. However, comparison of the measured pH and estimated bicarbonate concentration in the medullary loop structures and in the early distal tubule suggests that substantial bicarbonate absorption occurs in the loop of Henle beyond the proximal straight tubule [31, 58–60]. The mechanisms and magnitude of bicarbonate absorption in the individual segments of the loop of Henle have not yet been explored fully with isolated perfused tubules. Preliminary studies have not demonstrated any active bicarbonate absorption in these segments. Other mechanisms, such as back-titration of bicarbonate by buffers which were protonated in the proximal tubule or simple bicarbonate diffusion, could also be examined with the isolated, perfused tubule.

Thin descending limb. Bicarbonate reabsorption in the loop may result from continuing abstraction of water and concentration of luminal bicarbonate. If water abstraction simply increased the concentration of all luminal buffers, then the pH would not change. If the thin descending limb is permeable to carbon dioxide, then carbon dioxide loss would alkalinize the lumen and bicarbonate could be back-titrated by other buffers (for example, ammonium and monobasic phosphate) which had been protonated in the proximal tubule. Carbonic acid would thus be generated, and following its dehydration, carbon dioxide would diffuse out of the thin descending limbs into the medullary interstitium. The net effect would be a loss of bicarbonate in the form of carbon dioxide. On the other hand, bicarbonate could simply diffuse out of the thin descending limb once its concentration was elevated by water abstraction. The result of this process would be bicarbonate removal with acidification of the luminal fluid. The processes of back-titration and bicarbonate diffusion could result in loop bicarbonate absorption by passive mechanisms.

Several aspects of these reactions have been or could be examined at the level of the isolated tubule. Of primary importance is the demonstration that osmotic equilibration in the rabbit thin descending limb occurs by water abstraction rather than solute entry [61, 62]. In fact, the thin descending limb has the highest water permeability per surface area of any nephron segment, even exceeding that of the proximal tubule or the collecting tubule treated with antidiuretic hormone [63]. Therefore, osmotic water flow, driven by medullary hypertonicity, concentrates the luminal solutes including bicarbonate in the thin descending limb. Two factors determine whether back-titration reactions contribute to loop bicarbonate absorption. Firstly, carbon dioxide permeability of the thin descending limb could limit the extent of the back-titration reactions. If carbon dioxide were impermeable, then water abstraction would not result in back-titration of luminal bicarbonate and the lumen pH would not change. Any carbon dioxide which was generated would remain in the lumen, and total carbon dioxide removal (bicarbonate absorption) would be negligible. Secondly, protonated buffer species must be present to support the back-titration of luminal bicarbonate in response to water abstraction. The contributions of luminal back-titration reactions and simple

bicarbonate diffusion could be determined readily with the isolated perfused thin descending limb.

Thin ascending limb. Acidification mechanisms have not yet been characterized in this segment. The ascending limbs of the loop are extremely impermeable to water [63] so that water abstraction probably does not concentrate the luminal bicarbonate contents. The chloride permeability of the thin ascending limb is exceedingly high, and includes a large component of facilitated diffusion [64]. Exchange diffusion, either between halides or an exchange of chloride for bicarbonate, is not thought to occur in the thin ascending limb of the loop of Henle [64].

Thick ascending limb. Free-water clearance studies have shown that bicarbonate behaves like an impermeant anion in the thick ascending limb [65]. Acetazolamide increases bicarbonate delivery to the thick ascending limb but does not affect the generation of free water when expressed relative to the load of reabsorbable anion (for example, chloride). Furthermore, acetazolamide does not affect the lumen-positive transport potential or the rates of chloride reabsorption in isolated perfused thick ascending limbs [66]. Direct measurements in isolated perfused tubules have not demonstrated any bicarbonate reabsorption in the thick ascending limb [67, 68].

Recent studies of mouse cortical thick ascending limbs demonstrate zero net bicarbonate absorption in this segment but do indicate that bicarbonate and/or carbon dioxide play an important role in coupled sodium chloride transport across the luminal membrane [69]. In this regard, the stimulation of sodium chloride transport is similar to the effects of bicarbonate in other "leaky" epithelia [70]. Andreoli et al [69] have proposed that parallel Na^+/H^+ and Cl^-/HCO_3^- exchangers account in part for coupled sodium chloride cotransport in the mouse cortical thick ascending limb. Inhibitors of either exchanger (for example, amiloride or SITS) reduce net sodium chloride transport when examined in the presence of ambient bicarbonate and carbon dioxide [69]. It is not known if net bicarbonate absorption would be observed if only the anion exchanger was inhibited. If the rates of Na^+/H^+ and Cl^-/HCO_3^- exchange are matched exactly and tightly coupled, then net bicarbonate absorption would not be observable in this segment. On the other hand, if the parallel exchangers are coupled loosely as has been suggested for the proximal tubule [70–73], then it may be possible to demonstrate net bicarbonate reabsorption in the thick ascending limb while inhibiting the luminal anion exchanger with SITS. The physiologic significance of these results, and their applicability to species other than the mouse, remain to be established.

Distal convoluted tubule and connecting segment

These segments have not been evaluated with regard to acidification mechanisms in the isolated tubule preparation. There is a favorable electrical gradient for proton secretion (or bicarbonate absorption) in the rabbit distal convoluted tubule [74], but acidification rates have not yet been measured. Recent studies of the superficial distal convoluted tubule of the rat in normal acid-base states, did not reveal evidence for proton secretion [75] or for significant bicarbonate absorption [76]. Bicarbonate absorption was only observed during extreme systemic acidosis [76]. These results suggest that the distal convoluted tubule does not play an important role in acid-base homeostasis.

Collecting tubule

The final segments of the nephron play an important role in acid-base homeostasis. Their primary function is the titration of luminal buffers and delivery of new bicarbonate to the peritubular compartment. The overall result is net acid excretion and generation of bicarbonate to replace that which was consumed previously in buffering metabolic acid loads. Bicarbonate secretion in response to alkali loading is another aspect of collecting tubule function which may be especially important in herbivores that consume alkaline-ash diets. Whatever the balance between proton and bicarbonate secretions, there must be an overall regulation of the processes in the collecting tubule to maintain acid-base balance. Several factors appear to regulate collecting tubule function, including aldosterone status, distal delivery of sodium and volume, and systemic pH and potassium stores [31]. Recently, adaptation at the functional or structural level has been recognized as an important response to changes in acid-base status.

Cortical collecting tubule. H^+ secretion. The urinary bladders of toads and turtles have served as important models for the investigation of acidification mechanisms thought to pertain to the distal tubule. One of their salient features is the ability to secrete protons in the absence of sodium or during inhibition of sodium transport by amiloride or ouabain [77, 78]. Under these conditions, proton secretion is associated with a lumen-positive electrical potential and a reversed short-circuit current which can be inhibited by acetazolamide [77, 78]. Stoner, Burg, and Orloff [79] observed lumen-positive PD's when rabbit collecting tubules were exposed to luminal amiloride. These potentials were inhibited by acetazolamide and were, therefore, attributed to proton secretion in this segment [79]. (These findings have been confirmed with direct measurements of proton secretion by Koeppen and Helman [80].) Subsequently, Jacobson et al [81] observed lumen-positive PD's in human collecting tubules which could be inhibited by furosemide and were attributed to electrogenic chloride transport. Hanley et al [82] found that chloride replacement by methylsulfate abolished the lumen-positive PD in the collecting tubule, a finding which they interpreted as demonstrating electrogenic chloride transport. The discrepancy between the results of Stoner, Burg, and Orloff [79] and Koeppen and Helman [80], and those reported by Jacobson et al [81], and Hanley et al [82] is explained by the choice of anions substituting for chloride. Laski, Warnock, and Rector [83] have found that methylsulfate may inhibit bicarbonate transport in the cortical collecting tubule. Isethionate, cyclamate, or sulfate appear to be better choices for chloride-replacement studies. These considerations favor the previous interpretation that the lumen-positive PD is due to electrogenic proton transport in the cortical collecting tubule [79, 80].

The first direct measurements of bicarbonate transport in the rabbit cortical collecting tubule were reported by McKinney and Burg [84, 85]. They examined the relationships between bicarbonate and sodium transport, and divided their results into two groups. They observed that individual tubules would either secrete or absorb bicarbonate during the control periods, that dietary history affected the direction of bicarbonate transport, and that both secretion and absorption were inhibitable by

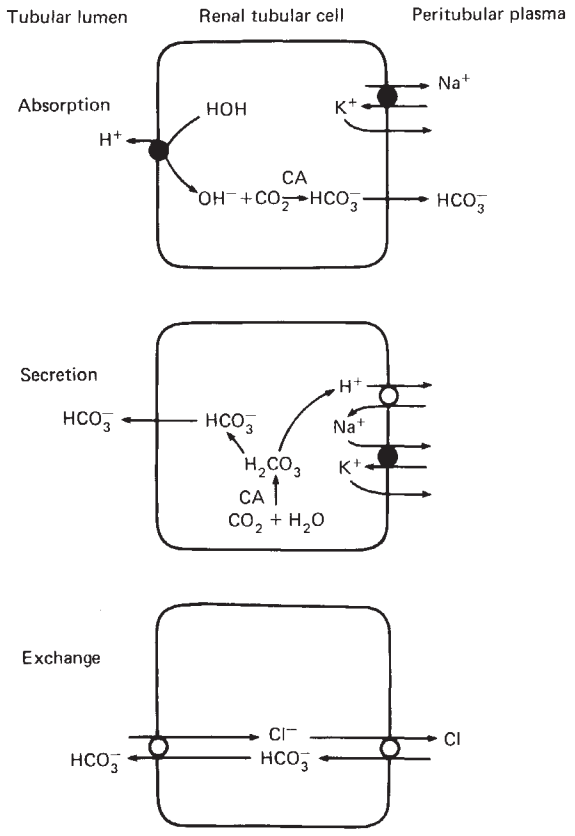

Fig. 2. *Possible mechanisms of hydrogen secretion and bicarbonate ion transport in the cortical collecting tubule. Open circles* denote ion exchange mechanisms driven by the respective chemical gradients. *Closed circles* denote active transport mechanisms. Bicarbonate absorption *(upper panel)* would be accomplished primarily by secretion of hydrogen into the lumen. An equivalent amount of bicarbonate would be generated in the cell and would cross the basolateral membrane via the bicarbonate exit step. If nonbicarbonate buffers are titrated by luminal hydrogen secretion, then new bicarbonate is delivered to the peritubular plasma compartment. Bicarbonate secretion *(middle panel)* could occur if the cellular bicarbonate ion content was elevated by a Na^+/H^+ exchanger in the basolateral membrane. The process would be electrogenic if a bicarbonate ion diffusive pathway existed in the luminal membrane. Bicarbonate exchange *(lower panel)* would require the presence of anion exchangers in both luminal and basolateral membranes.

acetazolamide [85]. Their results [84, 85] are consistent with separate, parallel pathways for bicarbonate absorption and secretion in which the net rate of transport is the sum of both processes.

A striking similarity between the rabbit cortical collecting tubule and the amphibian urinary bladder is the relationship between proton secretion and sodium transport. Amiloride will reduce net proton secretion in either preparation in association with reductions in the transepithelial PD [77, 84, 85]. This effect is due to hyperpolarization of the luminal membrane [86, 87], thereby increasing the unfavorable electrical gradient against which protons are secreted. A similar effect has been reported recently with lithium [88]. It is of interest that the proton secretory defect is repaired when the transepithelial PD is restored to the value observed before addition of amiloride [86, 89] or lithium [88]. These results indicate that amiloride or lithium do not directly inhibit proton secretion in amphibian bladders; the inhibition of proton secretion is secondary to the inhibition of the PD generated by sodium reabsorption. Similar results have been obtained recently in the isolated perfused cortical collecting tubule. Koeppen and Helman [80] measured luminal pH in the collecting perfusate and observed that ouabain reduced the lumen-negative PD and sodium transport, and also reduced luminal acidification. No effect of ouabain was observed if sodium transport was inhibited by omitting sodium. These results are entirely consistent with an indirect effect of ouabain on proton secretion resulting from its direct effect on sodium transport.

A characteristic of electrogenic proton secretion (Fig. 2, top panel) is a dependence on the transepithelial PD. Even though proton secretion is not coupled directly to sodium transport, it has been shown that the rate of acidification is increased in tubules with more lumen-negative transport PD's, either in association with ammonium chloride prefeeding [84] or chronic mineralocorticoid administration [80]. Koeppen and Helman have recently examined acidification and the associated lumen-positive PD observed in rabbit collecting tubules in the absence of sodium transport and found that both were stimulated by mineralocorticoids, were sensitive to the bath P_{CO_2}, and were reduced to zero by acetazolamide or SITS in the bathing media [80]. These results are identical to those obtained with amphibian systems [77, 78, 89–91] and strongly support the thesis that proton secretion is the primary mechanism of acidification in the rabbit cortical collecting tubule (Fig. 2, upper panel).

Another feature associated with electrogenic proton secretion by the amphibian urinary bladder [78] is the relatively low proton permeability. Recent studies [28] indicate that the proton permeability of the cortical collecting tubule is much lower than the proximal tubule, a necessary condition in view of the pH gradients which can be generated in either segment [31]. Low proton permeability reduces the passive collapse of pH gradients and would thereby increase the efficiency of net proton secretion by the collecting tubule.

HCO_3^- secretion and Cl^-/HCO_3^- exchange. McKinney and Burg described clear-cut bicarbonate secretion in isolated perfused cortical collecting tubules [84, 85]. This finding was accentuated in tubules taken from rabbits that had received isotonic sodium bicarbonate drinking water for at least 24 hr prior to sacrifice. Bicarbonate secretion was demonstrated when bath and initial perfusate contained identical bicarbonate concentrations (25 mM) and occurred against a lumen-negative PD. Therefore, bicarbonate secretion proceeded uphill against an unfavorable electrochemical gradient (Fig. 2, middle panel). Of note, this process was inhibited by acetazolamide and continued when the ambient chloride was replaced by sulfate, suggesting a primary bicarbonate secretory process rather than a coupled anion exchange process. Bicarbonate secretion also displayed a dependence on ambient sodium, a characteristic not readily explained by a primary active bicarbonate secretory process. Sachs, Faller, and Rabon [92] have proposed that a Na^+/H^+ exchanger could elevate cellular bicarbonate concentration in bicarbonate-secreting tissues. A bicarbonate exit pathway would have to exist in the luminal membrane to permit bicarbonate entry into the lumen. Bicarbonate secretion obviously limits net acidification of the lumen. Therefore, for net

acidification to proceed, the bicarbonate secretory mechanism must be inhibited. Either the bicarbonate secretory pathway or the basolateral Na^+/H^+ exchanger (Fig. 2, middle panel) could be regulated in response to changes in systemic acid-base balance.

In contrast to the finding of electrogenic bicarbonate secretion, the turtle bladder has been shown to have an anion-exchange mechanism by which bicarbonate is secreted into the mucosal solution by electrically-neutral exchange for chloride [93]. Of note, bicarbonate secretion in the turtle bladder does not display the sodium dependence [94] described for the rabbit cortical collecting tubule [85]. This finding suggests that the anion exchange is driven by the transepithelial chemical gradients for chloride and bicarbonate (Fig. 2, lower panel), rather than elevated cellular bicarbonate concentrations (Fig. 2, middle panel).

Further studies of the cortical collecting tubule by Boyer and Burg [95] and Laski, Warnock, and Rector [83] have demonstrated an apparent coupling between bicarbonate secretion and luminal chloride. Boyer and Burg [95] found that replacement of luminal chloride by sulfate decreased bicarbonate secretion in tubules which were perfused with solutions initially free of bicarbonate. Laski, Warnock, and Rector [83] extended these studies and found that transepithelial chloride gradients stimulated bicarbonate transport in the direction opposite to the applied chloride gradient. Of special interest, Laski, Warnock, and Rector [83] found that bicarbonate transport was affected by the imposition of a 50 mM chloride gradient, while the previous studies of Boyer and Burg [95] utilized complete replacement of chloride by sulfate. It is not known whether these findings represent an anion exchange mechanism in the cortical collecting tubule (Fig. 2, lower panel), or represent an electrical effect of chloride substitution on an electrogenic bicarbonate secretory system (Fig. 2, middle panel).

Recent studies of the turtle bladder [96] suggest an explanation for the similar chloride-bicarbonate exchanges in turtle bladder and rabbit cortical collecting tubule, and for the finding that bicarbonate secretion occurs in the cortical collecting tubule despite total removal of ambient chloride. Fischer, Husted, and Steinmetz [96] found that very low chloride concentrations were required to support bicarbonate exit, presumably by chloride-bicarbonate exchange, across the basolateral membrane of the turtle bladder. The apparent K_m for this process was 0.13 mM when chloride was replaced by gluconate. If a similar specificity pertains to a chloride-bicarbonate exchange at the luminal membrane of the cortical collecting tubule, then extreme care would be required to totally remove luminal chloride to examine the lack of chloride-requirement for bicarbonate secretion in the collecting tubule.

Medullary collecting tubule. Preliminary studies of the rabbit medullary collecting duct have shown that substantial rates of bicarbonate absorption can occur in this segment [97, 98]. The measured rates are nearly half those observed in the proximal straight tubule [3, 11] when expressed per unit length. The bicarbonate absorptive rates in the medullary collecting tubule exceed those of the cortical collecting tubule by at least a factor of three [85, 97, 98]. These findings indicate that the medullary collecting tubule plays an important role in acid-base homeostasis. Bicarbonate secretion or an effect of chronic alkali ingestion has not been reported in the medullary collecting tubule.

The PD in the medullary collecting tubule appears to be zero or positive [99]. In contrast to the cortical segment, sodium transport and stimulation of sodium transport by mineralocorticoids is not a prominent feature of the medullary collecting tubule. In contrast to the lack of mineralocorticoid effect on sodium transport, Stone, Kokko, and Jacobson [98] found that adrenalectomy reduces, and in vitro aldosterone replacement (10^{-6} M) stimulates bicarbonate absorption in the rabbit medullary collecting tubule. Of interest, proton secretion in this segment appears to be completely independent of sodium reabsorption [100], so that the stimulatory effect of aldosterone represents a direct effect on the proton secretory mechanism like that described with aldosterone in turtle bladders [101, 102]. Amiloride does not appear to inhibit proton secretion in this segment [100], in contrast to the effects of amiloride on sodium reabsorption and proton secretion in whole kidney studies [103–106], and in the collecting tubule [80]. These findings are consistent with electrogenic proton secretion in the medullary collecting tubule and with the lack of sodium transport at this site [99]. However, it is surprising that there is an acute in vitro effect of aldosterone on proton secretion [98] without an acute effect on the lumen-positive PD [100].

Papillary collecting duct. Isolated perfused segments of the papillary collecting duct have not yet been utilized for studies of acidification. Recent micropuncture studies have demonstrated bicarbonate reabsorption in the papillary collecting duct of the surgically exposed rat papilla [107]. DuBose has recently demonstrated proton secretion (that is to say, disequilibrium pH) with this preparation in bicarbonate-loaded animals [108]. Of note, the largest disequilibrium pH was observed at the earliest accessible site (papillary base) which may have been a reflection of the proton secretory rate in the upstream (medullary) segment of the collecting duct. This finding suggests that the rate of proton secretion may be greater in the medullary collecting duct than in the papillary collecting duct. Direct measurements of isolated perfused segments are required for the description of proton secretion by the papillary collecting duct.

Regulation of distal acidification

Studies of isolated perfused tubules have provided several important insights in the regulation of distal acidification. Of great importance is the recognition that the cortical collecting tubule can secrete protons or bicarbonate in response to the homeostatic requirements of the whole organism [84, 85]. While the bicarbonate secretory system is of obvious importance in a herbivore like the rabbit, recent studies [109] are also consistent with this system in the rat kidney from animals adapted to alkali-rich diets. Another aspect of the studies of McKinney and Burg [84, 85] deserves emphasis. They described a memory effect by which adaptations to in vivo pertubations of acid-base balance are carried over and expressed in the in vitro perfused collecting tubule. At the very least, this finding demonstrates that adaptive changes occur rapidly and imply that structural changes have taken place. Further insights into possible structural adaptations come from recent studies of the turtle bladder [110, 111]. Mitochondrial-rich cells contain carbonic anhydrase and represent the proton secretory population [110]. These cells may also contain an intracellular population of vesicles with hydrogen secretory systems [111]. Fusion of these vesicles with

the apical membrane could increase the density of hydrogen pumps in the apical membrane and thereby increase the rate of hydrogen secretion. This phenomena has been observed in turtle bladders in response to hypercapnia [111] and in the intercalated cells of the rabbit cortical collecting tubule in response to chronic aldosterone administration [112].

A final issue concerns the functional differences between the cortical and medullary collecting tubules. The intrinsic proton secretory capacity of the medullary collecting tubule [97, 98] exceeds that of the cortical collecting tubule [84], possibly accounting for the progressive acidification of the urine from cortex to medulla. Furthermore, the response to aldosterone differs between the two segments. Aldosterone directly stimulates sodium reabsorption in the cortical collecting tubule and indirectly stimulates hydrogen secretion due to its effect on transepithelial PD [80]. Amiloride decreases the transepithelial sodium transport PD and thereby diminishes the rate of proton secretion [80, 84] in the cortical collecting tubule. Proton secretion is stimulated directly by aldosterone in the medullary collecting tubule, apparently without a secondary effect due to stimulation of sodium transport [98, 100]. These functional differences may localize defects in distal hydrogen secretion. In those conditions in which distal acidification can be stimulated by distal delivery of sodium with an impermeant anion [113, 114], the cortical collecting tubule would appear to be intact. On the other hand, the failure to increase distal proton secretion in response to increases in distal sodium avidity could be explained by defective proton secretion in the medullary collecting tubule or by a more generalized defect in both cortical and medullary segments.

Note added in proof

The reference mentioned in the text as *Sasaki, Berry, and Rector, to be published* has been accepted for publication: SASAKI S, BERRY CA, RECTOR FC JR: Effect of acute changes in bath potassium concentration on bicarbonate absorption in the rabbit proximal convoluted tubule. *Am J Physiol* in press, 1982.

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